

ADJUVANT PROPERTIES IN HUMAN DIALYSABLE LEUKOCYTE
EXTRACTS (DLE) CONTAINING TRANSFER FACTOR

A. O. Anderson, M. S. Ascher and L. A. Andron
U. S. Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701

Agents which intensify traffic of recirculating small lymphocytes through lymphatic tissues may be predicted to have adjuvant activity since more potentially responsive immunocytes will be exposed to antigen and there is increased opportunity for cellular cooperation (1,2).

It has been suggested that dialysable leukocyte extracts (DLE) might produce their effects in vivo through a mechanism similar to adjuvants (3). DLE are chemotactic for leukocytes including lymphocytes (4), and nonspecifically augment antigen-dependent lymphocyte proliferation in vitro (5). Present studies show that when DLE are injected into afferent lymphatic beds, they appear to enhance the regional lymph node response to antigen.

I. MATERIALS AND METHODS

Human DLE were prepared as reported previously (6) and 0.1 ml, derived from 5×10^5 lymphocytes, was injected subcutaneously into the right lateral thorax or foot pad of Lewis rats and Hartley guinea pigs. Minimum essential medium for suspension cultures (MEM) was injected contralaterally as a control. At 24 hours, the regional and contralateral lymph nodes were excised, stripped of fat, and weighed. The nodes were fixed and subsequently examined by light microscopy. Percent cortical area was calculated using planimetry, and the rate of lymphocyte entry was evaluated using the lymphocyte migration index (LMI), calculated as the number of migrating lymphocytes in the wall of a high endothelial venule (HEV) divided by the number of endothelial cells in that cross-section.

Guinea pigs were injected with killed antigen derived from Francisella tularensis alone or combined with DLE or MEM. At two-week intervals the guinea pigs were bled via cardiac puncture and their white cells cultured with tularemia antigen. The cellular response over 5 days in culture was quantitated as CPM [^{14}C]thymidine incorporated. At nine weeks skin tests were performed with tularemia antigen.

II. RESULTS

Fig. 1. Local subcutaneous injection of DLE caused rapid enlargement of regional (axillary) nodes in the rat at 24 hours when compared to the contralateral control node injected with MEM. A dose response showed persistence of the effect at 10^{-4} dilution. The abscissa is relative DLE concentration and the error bars are \pm S.E.M.

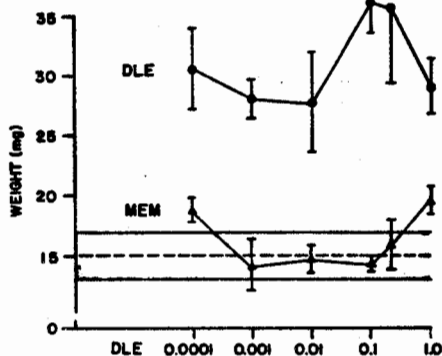


Fig. 2. The lymphocyte migration index (LMI) increased in nodes on the DLE side. These data were corroborated by autoradiographic studies using intravenous infusion of ^3H uridine labeled thoracic duct lymphocytes.

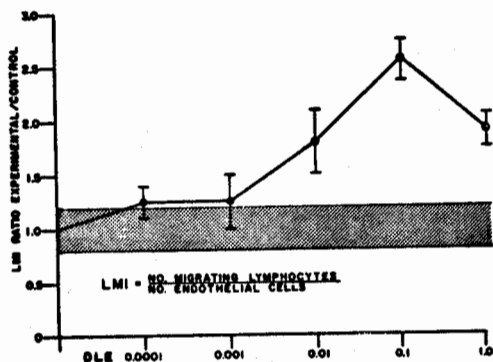
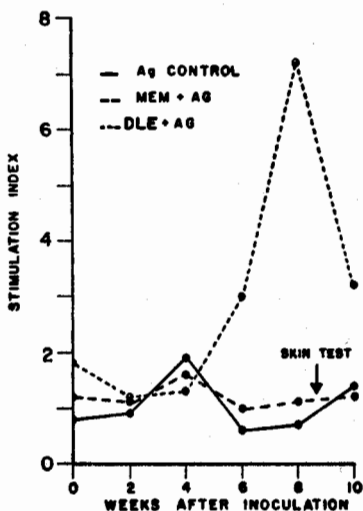


Fig. 3. Regional injection of DLE in combination with killed microbial antigen gave increased lymphocyte transformation and delayed skin reactivity when compared to animals receiving antigen alone, DLE alone or antigen with MEM.

Morphological observations in DLE-draining lymph nodes included: planimetric evidence of deep cortical expansion, distension of intermediary sinuses with small lymphocytes, "activation" of sinusoidal macrophages and focal mast cell degranulation.



III. DISCUSSION

The induction of immunity requires an intricate system of cooperative cellular interactions among classes of lymphocytes and adherent cells. An appreciation of the cellular requirements in immune induction has evolved from in vitro studies (7). However, one must return to in vivo models to study factors which regulate the natural immune response, since in vitro systems do not necessarily reflect the dynamic milieu of normal lymphatic tissues (8). Percolation of recirculating lymphocytes through sites of antigen concentration provides a natural environment for clonal selection (9). Nonspecific and specific alterations in lymphocyte traffic through lymph nodes are known to occur following antigenic stimulation (10-12), and qualitative differences in the ability of certain antigens to produce lymphocyte traffic changes appear to correlate with the immunogenicity of the antigen (8). Adjuvants may potentiate the immune response to weak antigens by causing prolonged increases in lymphocyte traffic and augmenting proliferation of antigen sensitized cells(1,2). DLE appear to have these properties, since rapid node enlargement, paracortical expansion and increased lymphocyte traffic across HEV walls were seen in rat lymph nodes draining injections of DLE. The ability of leukocyte extracts to augment response to antigen was also evident peripherally where DLE enhanced lymphocyte transformation and delayed-type hypersensitivity reactions in guinea pigs. Thus, the immunopotential observed with DLE in these animal models appears similar to that produced by recognized adjuvants.

IV. REFERENCES

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